

Access DB# _____

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: JANE ZARA Examiner #: 77512 Date: 11/
An Unit: 1635 Phone Number 306-5820 Serial Number: 09/880,821
Mail Box and Bldg. Room Location: 11D03 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc. if known. Please attach a copy of the cover sheet, pertinent claims, and abstract

Title of Invention: Polysynaptic Nuclear Acids

Inventors (please provide full names): Eyles et al.

Earliest Priority Filing Date: 7/14/00

For Sequence Searches Only Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

Please Search Seq ID# 142.

For regular data
bases + references.

Length unlimited.
Limit to 100 NT.

Thank you

RNA was isolated from the transfected cells as described in Section 1 and PCR was carried out to identify cellular CCR5 or CXCR4 mRNA using the above-described CCR5 or CXCR4 primer. Also, as the cells naturally express actin, the amount of mRNA of actin provides a quantitative control. Such a control confirms that the inhibition of CCR5 and/or CXCR4 is not due to the general degradation of RNA; otherwise the actin RNA would have degraded too. It also confirms that the ribozyme action is specific, in that it does not cleave actin RNA. This control, using actin, is widely applied in molecular biology.

The results are shown in Figures 13a, 13b and 14 which are stained agarose gel photographs of the relevant PCR products. Figs. 13a and 13b relate, respectively, to the first and second sets of experiments. Fig. 13a showing the action of the ribozyme against CCR5 RNA and 13b showing the action against CXCR4 RNA. The arrangement of Figures 13a and 13b is the same, and is as follows, numbering the lanes 1-8 from left to right:

| mRNA type | Lane No. | Polymerase vector present in vector system | Autopolymerase vector present in vector system | Ribozymal DNA vector present in vector system |
|---------------|----------|--|--|---|
| CCR5 or CXCR4 | 1 | Yes | Yes | Yes |
| " | 2 | Yes | No | Yes |
| " | 3 | Yes | No | No |
| " | 4 | [Molecular weight markers] | | |
| Actin | 5 | Yes | Yes | Yes |
| " | 6 | Yes | No | Yes |
| " | 7 | Yes | No | No |
| " | 8 | [Molecular weight markers] | | |

93
11/25/03

Lane 1 shows that CCR5 and mRNA was not ~~detectable~~ ^{detectable} from the PBMC transfected with all three vectors, i.e. the polymerase, the autopolymerase and the ribozymal DNA vectors, thus indicating complete inhibition of CCR5 mRNA. Lane 2 contains a weak band of CCR5 mRNA, showing that without the autopolymerase vector, the inhibition of CCR5 and CXCR4 mRNA was incomplete. Lane 3 contains a bright band of the CCR5 or CXCR4 RNA, showing that without the ribozyme vector